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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
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EXAMINER	
BERTAGNA, ANGELA MARIE	
ART UNIT	**PAPER NUMBER**
1637	

DATE MAILED: 07/20/2006

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/777,691	NGUYEN, KHUE VU	
	Examiner	Art Unit	
	Angela Bertagna	1637	

-- *The MAILING DATE of this communication appears on the cover sheet with the correspondence address* --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE _3_ MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☐ Responsive to communication(s) filed on _____.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) _1-3_ is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) _1-3_ is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☒ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on _15 June 2004_ is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

1. An examination of this application reveals that applicant is unfamiliar with patent prosecution procedure. While an inventor may prosecute the application, lack of skill in this field usually acts as a liability in affording the maximum protection for the invention disclosed. Applicant is advised to secure the services of a registered patent attorney or agent to prosecute the application, since the value of a patent is largely dependent upon skilled preparation and prosecution. The Office cannot aid in selecting an attorney or agent.

A listing of registered patent attorneys and agents is available on the USPTO Internet web site http://www.uspto.gov in the Site Index under “Attorney and Agent Roster.” Applicants may also obtain a list of registered patent attorneys and agents located in their area by writing to the Mail Stop OED, Director of the U. S. Patent and Trademark Office, PO Box 1450, Alexandria, VA 22313-1450

Information Disclosure Statement

2. The listing of references in the specification is not a proper information disclosure statement. 37 CFR 1.98(b) requires a list of all patents, publications, or other information submitted for consideration by the Office, and MPEP § 609.04(a) states, "the list may not be incorporated into the specification but must be submitted in a separate paper." Therefore, unless the references have been cited by the examiner on form PTO-892, they have not been considered.

Specification

3. The abstract of the disclosure is objected to because it consists of three paragraphs instead of the required single paragraph. Correction is required. See MPEP § 608.01(b).

Claim Rejections - 35 USC § 112

4 The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 1-3 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 1 is rejected under 35 U.S.C. 112, second paragraph, as being incomplete for omitting essential steps, such omission amounting to a gap between the steps. See MPEP § 2172.01. The omitted steps are: cloning of the SMN gene. The method only recites reverse transcription followed by PCR with no additional steps related to the stated goal of the method - cloning the human SMN gene.

Claims 2 and 3 contain the trademark/trade names pFastBac™, DH10Bac™, and pCR®II. Where a trademark or trade name is used in a claim as a limitation to identify or describe a particular material or product, the claim does not comply with the requirements of 35 U.S.C. 112, second paragraph. See *Ex parte Simpson*, 218 USPQ 1020 (Bd. App. 1982). The claim scope is uncertain since the trademark or trade name cannot be used properly to identify any particular material or product. A trademark or trade name is used to identify a source of goods, and not the goods themselves. Thus, a trademark or trade name does not identify or

describe the goods associated with the trademark or trade name. In the present case, the trademark/trade name is used to identify/describe a vector and competent cells, respectively, and, accordingly, the identification/description is indefinite.

It is also noted that claims 2 and 3 use the terms "pBlueBacHis2A", "INV αF'", and "pET-28a(+)". Although these terms are not identified in the claims with the "TM" symbol, these are also trademarks/trade names for vectors and competent cell lines.

Claim Rejections - 35 USC § 103

5. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

6. Claim 1 is rejected under 35 U.S.C. 103(a) as being unpatentable over Wirth et al. (American Journal of Human Genetics (1999) 64: 1340-1356) in view of Lefebvre et al. (Cell (1995) 80: 155-165) and further in view of Powell (PCR Protocols: A Guide to Methods and Applications (1990) by Academic Press, Inc. pages 237-243) and further in view of Buck (Biotechniques (1999) 27(3): 528-36) and further in view of Innis et al. (PCR Protocols: A Guide to Methods and Applications (1990) by Academic Press, Inc. pages 3-12).

Wirth teaches a method comprising RT-PCR of the human SMN1 gene and cloning of the resulting products (see pages 1342-1343).

Specifically, the method of Wirth comprises:

(a) isolating RNA (page 1342 "RNA isolation and RT-PCR section")

(b) performing an RT reaction using oligo-dT primers under the conditions specified by Life Technologies for use with the M-MLV reverse transcriptase (65°C denaturation for 5 minutes, quick chill on ice, room temperature (approximately 25°C) for 10 minutes, and reverse transcription at 37°C for 50 minutes – see page 1342 "RNA isolation and RT-PCR" section and also the attached product information sheet from Life Technologies)

(c) performing PCR using two specific oligonucleotide primers where the reaction conditions comprise: 94°C for 2 min followed by 30 cycles of 15 sec at 94°C, 30 sec at 67°C, and 1 min at 72°C, with the elongation time being extended by 20 seconds in each cycle beginning at cycle 11 (page 1342 "RNA isolation and RT-PCR" section).

Wirth further teaches that these RT-PCR products were cloned into the pCRII-TOPO cloning vector (page 1343, "Cloning of PCR products and sequencing").

Wirth teaches reverse transcription using oligo-dT primers rather than the instantly claimed SEQ ID No: 1 using slightly different reaction conditions than those instantly claimed. Specifically, Wirth teaches: (a) denaturation at 65°C for 5 minutes rather than denaturation at 90°C for 5 minutes, (b) a quick chill on ice without a specific incubation time, and (c) reverse transcription at 37°C for 50 minutes rather than at 42°C for 45 minutes.

Also, the PCR of Wirth utilizes different primers than the instantly claimed SEQ ID Nos: 2 and 3 and slightly different reaction conditions. Specifically, Wirth teaches: (a) denaturation at 94°C for 2 minutes initially followed by 15 seconds in subsequent cycles rather than 1 minute

denaturations in all cycles, (b) annealing at 67°C for 30 seconds rather than at 55°C for 2 minutes, and (c) elongating for an additional 20 seconds in each cycle following cycle 11. Also, Wirth performs 30 cycles of PCR amplification rather than the 35 cycles of claim 1.

Lefebvre teaches the nucleic acid sequence of the human SMN1 gene (Figure 3, page 158).

Powell teaches that reverse transcription may be performed using oligo-dT primers or a specific primer (page 243). Powell further states, "One of the advantages of using specific primers close to the site of modification is that cDNA synthesis and subsequent amplification may be performed on les than intact preparations of total RNA" (page 243).

Buck analyzed the effect of primer design strategy on the performance of DNA sequencing primers. Specifically, Buck invited primer submissions from a number of labs (39) (page 532, column 3), with 69 different primers being submitted (see page 530, column 1). Buck also tested 95 primers spaced at 3 nucleotide intervals along the entire sequence at issue, thereby testing more than 1/3 of all possible 18 mer primers on the 300 base pair sequence (see page 530, column 1). When Buck tested each of the primers selected by the methods of the different labs, Buck found that every single primer worked (see page 533, column 1). Only one primer ever failed, No. 8, and that primer functioned when repeated. Further, every single control primer functioned as well (see page 533, column 1). Buck expressly states "The results of the empirical sequencing analysis were surprising in that nearly all of the primers yielded data of extremely

high quality (page 535, column 2)." Therefore, Buck provides direct evidence that all primers would be expected to function, and in particular, all primers selected according to the ordinary criteria, however different, used by 39 different laboratories. It is particularly striking that all 95 control primers functioned, which represent 1/3 of all possible primers in the target region. This clearly shows that every primer would have a reasonable expectation of success.

Innis teaches general conditions for PCR amplification. These conditions are: 25 – 35 cycles of 96°C for 15 seconds (usually with a longer initial denaturation step), 55°C for 30 seconds, and 72°C for 1.5 minutes (page 4). Innis expressly states, "It can be highly advantageous to optimize the PCR for a given application" (page 4). Innis further teaches on pages 7-9, that parameters such as the time and/or temperature of the denaturation, annealing, and extension steps, as well as the number of cycles should be optimized based on the specific properties (base composition, length, concentration) of the target and primer sequences.

It would have been prima facie obvious for one of ordinary skill in the art at the time of invention to combine the teachings of Wirth, Lefebvre, Powell, Buck and Innis. Wirth expressly taught cloning of RT-PCR products generated from the human SMN gene. Since Powell taught that a specific reverse transcription primer permitted cDNA synthesis and subsequent amplification from less than intact RNA preparations (see above), the ordinary practitioner would have been motivated to substitute a specific RT primer for the oligo-dT primer of Wirth in order to promote more robust, accurate cDNA synthesis. Furthermore, since the SMN gene sequence was known, as evidenced by Lefebvre (cited above), and since Buck demonstrated the

equivalence of different primer sequences (see above), the person of ordinary skill would have been motivated to select any desired specific reverse transcription and PCR amplification primers based on the known SMN gene sequence to clone the gene expecting a reasonable level of success.

Regarding the selection of particular primer sequences, attention is also directed to the court decision *In Re Deuel* 34 USPQ 2d 1210 (Fed. Cir. 1995) where the Court of Appeals for the Federal Circuit determined that the existence of a general method of identifying a specific DNA does not make the specific DNA obvious. Regarding structural or functional homologues, however, the Court stated,

"Normally, a *prima facie* case of obviousness is based upon structural similiarity, i.e., an established structural relationship between a prior art compound and the claimed compound. Structural relationships may provide the requisite motivation or suggestion to modify known compounds to obtain new compounds. For example, a prior art compound may suggest its homologs because homologs often have similar properties and therefore chemists of ordinary skill would ordinarily contemplate making them to try to obtain compounds with improved properties".

As noted above, the human SMN sequence was well known in the art at the time of invention, as evidenced by Figure 3 of Lefebvre. Since the claimed primers simply represent structural homologues, which were derived from a sequence suggested by the prior art as useful for amplification primers, and concerning which a biochemist of ordinary skill would attempt to obtain alternate compounds with improved properties, the claimed primers are *prima facie* obvious over the cited references in the absence of secondary considerations.

Finally, since Innis taught that PCR amplification conditions should be optimized based on the base composition, length, and concentration of the target and primer sequences (see page 4 and 7-9, cited above), the ordinary practitioner would have motivated to optimize the thermal

cycling profile in order to maximize product yield without compromising reaction specificity. Attention is further directed to the court decision *In re Aller*, 105 USPQ 233 at 235:

> More particularly, where the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation.

Routine optimization is not considered inventive and no evidence has been presented that the selection of the claimed RT and PCR reaction conditions was other than routine or that the results should be considered unexpected in any way as compared to the closest prior art.

Therefore, the person of ordinary skill in the art, interested in obtaining a human SMN RT-PCR product, would have been motivated to perform the RT-PCR method taught by Wirth using any primer set derived from the known human SMN gene sequence, as suggested by Powell, Lefebvre, and Buck, under optimized reaction conditions, as suggested by Innis, thus resulting in the instantly claimed method.

7. Claim 1 is rejected under 35 U.S.C. 103(a) as being unpatentable over Nguyen et al. (US 2003/0049627) in view of Lefevre et al. (Cell (1995) 80: 155-165) and further in view of Buck et al. (Biotechniques (1999) 27(3): 528-36) and further in view of Innis et al. (PCR Protocols: A Guide to Methods and Applications (1990) by Academic Press, Inc. pages 3-12).

Nguyen teaches an RT-PCR method of cloning the human SMN gene (paragraphs 30-38).

Briefly, the method of Nguyen comprises:

(a) isolating RNA (paragraphs 29-30)

(b) performing an RT reaction using a specific oligonucleotide sequence based on the known SMN gene – CACATTGCATTTG (paragraph 32) under the following reaction conditions: 90°C for 2 minutes, cooling on ice 1 minute, 25°C for 10 minutes, 42°C for 45 minutes (paragraph 33)

(c) performing PCR using two synthesized oligonucleotides (SEQ ID Nos: 3-4 of Nguyen) under the following conditions: denaturation at 94°C for 1 minute; annealing at 55°C for 2 minutes, elongation at 72°C for 1 minute for 25 cycles (paragraph 35). Nguyen also taught PCR amplification of the SMN gene using the sequence: TCCTTAATTTAAGGAATGTGA (sequence g in paragraph 38). This sequence (sequence g of the Nguyen publication) overlaps with the instant SEQ ID No: 3 in 17/21 nucleotides. The differences between sequence g of Nguyen and the instant SEQ ID No: 3 are: (a) the presence of an additional three nucleotides at the 5' end in sequence g of Nguyen and (b) the absence of the four 3' terminal nucleotides of the instant SEQ ID No: 3 (GCAC) in sequence g of Nguyen.

To summarize, Nguyen teaches designing the RT and PCR primers from the known human SMN gene sequence (paragraphs 30-38), and also teaches a primer highly similar to the instant SEQ ID No: 3 (see above). However, Nguyen does not teach RT-PCR using the instant SEQ ID No: 1 for the RT reaction and SEQ ID Nos: 2 & 3 for the PCR. Also, although the reaction conditions of the Nguyen PCR are highly similar to the instant claim, Nguyen teaches 25 cycles rather than the instantly claimed 35 cycles.

Lefebvre teaches the nucleic acid sequence of the human SMN1 gene (Figure 3, page 158).

Buck analyzed the effect of primer design strategy on the performance of DNA sequencing primers. Specifically, Buck invited primer submissions from a number of labs (39) (page 532, column 3), with 69 different primers being submitted (see page 530, column 1). Buck also tested 95 primers spaced at 3 nucleotide intervals along the entire sequence at issue, thereby testing more than 1/3 of all possible 18 mer primers on the 300 base pair sequence (see page 530, column 1). When Buck tested each of the primers selected by the methods of the different labs, Buck found that every single primer worked (see page 533, column 1). Only one primer ever failed, No. 8, and that primer functioned when repeated. Further, every single control primer functioned as well (see page 533, column 1). Buck expressly states "The results of the empirical sequencing analysis were surprising in that nearly all of the primers yielded data of extremely high quality (page 535, column 2)." Therefore, Buck provides direct evidence that all primers would be expected to function, and in particular, all primers selected according to the ordinary criteria, however different, used by 39 different laboratories. It is particularly striking that all 95 control primers functioned, which represent 1/3 of all possible primers in the target region. This clearly shows that every primer would have a reasonable expectation of success.

Innis teaches general conditions for PCR amplification. These conditions are: 25 – 35 cycles of 96°C for 15 seconds (usually with a longer initial denaturation step), 55°C for 30 seconds, and 72°C for 1.5 minutes (page 4). Innis expressly states, "It can be highly

advantageous to optimize the PCR for a given application" (page 4). Innis further teaches on pages 7-9, that parameters such as the time and/or temperature of the denaturation, annealing, and extension steps, as well as the number of cycles should be optimized based on the specific properties (base composition, length, concentration) of the target and primer sequences. Regarding the number of cycles, Innis states, "The optimum number of cycles will depend mainly upon the starting concentration of target DNA when other parameters are optimized" (page 8). Innis further provides a table of with guidance as to how many cycles should be used based on the initial concentration of the target sequence (see page 9).

It would have been prima facie obvious for one of ordinary skill in the art at the time of invention to perform the RT-PCR amplification of the human SMN gene taught by Nguyen using any desired primer set under optimized reaction conditions. Nguyen expressly taught cloning of RT-PCR products generated from the human SMN gene using a specific primers in both the RT and PCR steps designed based on the known SMN gene sequence (see above, in particular, paragraphs 32, 33, and 35). Since the SMN gene sequence was known, as evidenced by Lefebvre (cited above), and since Buck demonstrated the equivalence of different primer sequences (also see above citation), the person of ordinary skill would have been motivated to select any desired specific reverse transcription and PCR amplification primers based on the known SMN gene sequence to clone the gene expecting a reasonable level of success.

Regarding the selection of particular primer sequences, attention is also directed to the court decision *In Re Deuel* 34 USPQ 2d 1210 (Fed. Cir. 1995) where the Court of Appeals for the Federal Circuit determined that the existence of a general method of identifying a specific

DNA does not make the specific DNA obvious. Regarding structural or functional homologues, however, the Court stated,

"Normally, a *prima facie* case of obviousness is based upon structural similiarity, i.e., an established structural relationship between a prior art compound and the claimed compound. Structural relationships may provide the requisite motivation or suggestion to modify known compounds to obtain new compounds. For example, a prior art compound may suggest its homologs because homologs often have similar properties and therefore chemists of ordinary skill would ordinarily contemplate making them to try to obtain compounds with improved properties".

As noted above, the human SMN sequence was well known in the art at the time of invention, as demonstrated by Figure 3 of Lefebvre. Since the claimed primers simply represent structural homologues, which were derived from a sequence suggested by the prior art as useful for amplification primers (recall that sequence g of Nguyen is highly similar to the instant SEQ ID No: 3, discussed above), and concerning which a biochemist of ordinary skill would attempt to obtain alternate compounds with improved properties, the claimed primers are *prima facie* obvious over the cited references in the absence of secondary considerations.

Finally, regarding the difference in reaction conditions, since Innis taught that the number of PCR cycles should be optimized based on the concentration of the target sequence (see pages 8-9, cited above), the ordinary practitioner would have motivated to optimize the number of cycles in order to maximize product yield without compromising reaction specificity. Attention is further directed to the court decision *In re Aller*, 105 USPQ 233 at 235:

> More particularly, where the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation.

Routine optimization is not considered inventive and no evidence has been presented that the selection of the claimed PCR reaction conditions (35 cycles versus 25 cycles in the prior art) was other than routine or that the results should be considered unexpected in any way as compared to the closest prior art.

Therefore, the person of ordinary skill in the art, interested in obtaining a human SMN gene RT-PCR product, would have been motivated to perform the human SMN RT-PCR method taught by Nguyen using any primer set derived from the known human SMN gene sequence, as suggested by Lefebvre and Buck, under optimized reaction conditions, as suggested by Innis, thus resulting in the instantly claimed method.

8. Claim 2 is rejected under 35 U.S.C. 103(a) as being unpatentable over Melki et al. (USPN 6,080,577) in view of the Invitrogen TOPO TA cloning manual, version N (copyright 1999-2001) and further in view of the Invitrogen Bac-to-Bac Baculovirus Expression System product information sheet (published 2002 by Invitrogen) and further in view of the Invitrogen pBlueBacHis2 A, B, and C product information sheet, version G (published Feb. 7, 2003), and further in view of the 1997 Invitrogen catalog (published 1997, page 85) and further in view of Sambrook et al. (Molecular cloning: a laboratory manual (1989) by Cold Spring Harbor, N.Y.: Cold Spring Harbor Laboratory, pages 1.53-1.61).

Melki teaches construction of SMN expression plasmids (see column 3, lines 56-60).

Melki teaches that the SMN gene may be expressed in "plasmids, cosmids, phages, YAC, pYAC, and the like" (column 13, lines 39-42). Melki further teaches expression in baculovirus cells (column 13, lines 49-50).

Melki teaches general methods for transferring the SMN gene between vectors comprising restriction digestion and ligation into a bacteriophage vector, and sequencing with M13 primers (see, for example, Example 6, column 28, lines 30-59).

However, Melki does not transfer the SMN gene from the pCR®II vector to the pFastBac vector followed by transformation into INVαF' and DH10Bac cells, colony screening and sequencing.

The Invitrogen Bac-to-Bac Baculovirus Expression System product information teaches that this expression system offers: "(a) tremendous time savings over traditional baculovirus methods, (b) high-level recombinant protein yields, (c) easy cloning (see page 1)." This product information sheet teaches that the pFastBac™ vector offers "high-level, native protein expression and a large multiple cloning site for simplified cloning" (page 3). This product information sheet further teaches that the multiple cloning site has BamHI and XhoI sites (see Figure 4). Finally, the product information sheet teaches transformation of the recombinant pFastBacTM construct into DH10Bac competent E. coli followed by blue-white screening for inserts (see page 5 and Figure 6).

This product information sheet does not teach screening in INVαF' cells or generating an expression construct using the pBlueBacHis2 A™ vector. Also, this product information sheet does not teach that the construct is generated by digesting the gene of interest from the pCR®II vector.

The Invitrogen pBlueBacHis2 A, B, and C product information sheet teaches that these baculovirus transfer vectors are designed for expression and purification of recombinant proteins in insect cells (page 1). This product information sheet states, "The histidine residues create a high-affinity metal binding site to allow purification of recombinant fusion proteins on nickel-chelating resin" (page 1). This reference teaches that the vector has BamHI and XhoI sites in the multiple cloning site (see page 3), and further teaches that the vector has blue-white screening capabilities (see Appendix, page 11).

Similar to the previous reference, this product information sheet does not provide specific details of the method of transferring a gene in a pFastBac™ vector to the pBlueBacHis2 A vector, such as alkaline phosphatase treatment or use of a specific cloning vector (pCR®II). Rather, the user is directed to Sambrook et al. (Molecular cloning: a laboratory manual, 1989) or other similar references for guidance (see page 2). Also, although this reference recommends transformation into a recA and endonuclease A deficient strain and provides several examples of suitable bacterial strains, INVαF' cells are not explicitly taught (page 2).

The 1997 Invitrogen catalog (page 85) teaches INVαF' competent E. coli cells. This strain is recA and endonuclease A deficient. Invitrogen teaches that these cells "are ideal for propagating plasmids and cDNA library construction. They allow stable replication of high-copy plasmids" (page 85). The genotype offers the following features: (a) blue-white screening, (b) reduction of homologous recombination of transformed plasmids (recA), and (c) increased quality of plasmid DNA preparations (endA1) (see page 85).

The Invitrogen TOPO TA cloning manual (published 2001) teaches that the pCR®II-TOPO vector provides "a highly efficient, 5 minute, one-step cloning strategy for the direct insertion of Taq polymerase PCR amplified products into a plasmid vector. No ligase, post-PCR procedures, or PCR primers containing specific sequences are required" (page 1). This vector contains BamHI and XhoI sites in the cloning site (see page 10).

Sambrook teaches a general protocol for cloning in plasmid vectors. Sambrook teaches that genes may be transferred between different vectors (such as a cloning and expression vector) via restriction enzyme digestion to release the fragment of interest followed by ligation into the new vector and transformation into competent E. coli (page 1.53-1.54; see also Figure 1.6). Sambrook teaches that digestion with two different restriction enzymes is desirable, because the background of nonrecombinant clones is low and the insert is only capable of ligation in one orientation (see Table 1.2). Sambrook teaches that the vector must also be digested using the same restriction enzymes used to release the insert, followed by alkaline phosphatase treatment to prevent re-circularization of the vector (see Figure 1.8 and pages 1.60-1.61, where calf intestinal alkaline phosphatase is taught).

It would have been prima facie obvious for one of ordinary skill in the art at the time of invention to construct expression plasmids of the human SMN gene in either the FastBac or BlueBacHis2A expression vectors according to the claimed procedure. Melki taught expression constructs of the human SMN gene using a variety of different vectors, including baculovirus vectors (see above), but did not specify a preferred baculovirus expression vector system. As

discussed above, the FastBac and BlueBacHis2A systems offered the user rapid, stable expression of recombinant proteins. The BlueBacHis2A system further afforded the ability to rapidly and efficiently purify the expressed protein using a histidine affinity tag (see above product information sheet). The ordinary practitioner would have been motivated to generate SMN expression constructs as suggested by Melki using the baculovirus vectors taught by Invitrogen in order to rapidly produce a high yield of recombinant protein, capable of being purified using simple, often one-step affinity-based methods. Since the Invitrogen product information sheets provided specific guidance as to the method of producing the expression construct, and Sambrook taught specific details of molecular cloning, the ordinary practitioner would have expected a reasonable level of success in producing the claimed expression constructs. Finally, regarding the choice of cloning host cells, the Invitrogen product information sheet for pBlueBacHis2A taught that the major requirement for the host cell is a strain deficient in recA and endA activity, and further taught that in addition to the exemplary E. coli strains mentioned on page 2, other equivalent strains could be used. Since the Invitrogen catalog taught that INVαF' cells were recA and endA deficient and useful for routine cloning applications (see above), the ordinary practitioner would have been motivated to substitute any suitable recA endA deficient cell line in the method. In short, the claimed method is directed to obtaining an expression construct of a known gene (SMN) in a known expression system (FastBac or BlueBacHis2A) using well-known, standard methods, and therefore is prima facie obvious in light of the prior art teachings cited above.

9. Claim 3 is rejected under 35 U.S.C. 103(a) as being unpatentable over Melki et al. (USPN 6,080,577) in view of the Novagen pET system manual (published February 1999) and further in view of Novagen catalog No. TB074 (December, 1998) and further in view of the 1997 Invitrogen catalog (published 1997, page 85).

Melki teaches general methods for transferring the SMN gene between vectors comprising restriction digestion and ligation into a bacteriophage vector, and sequencing with M13 primers (see, for example, Example 6, column 28, lines 30-59).

Melki teaches many different expression systems including baculovirus and bacterial expression vectors (column 13, lines 39-42), but does not specificy the pET-28a(+) bacterial expression vector. Also, Melki does not teach transfer the SMN gene from the pFastBac vector followed by transformation into INVαF' cells and colony screening.

The pET system is a bacterial expression system developed by Novagen. The pET system manual states, "The pET system is the most powerful system yet developed for the cloning and expression of recombinant proteins in E. coli. Target genes are cloned in pET plasmids under control of strong bacteriophage T7 transcription and (optionally) translation signals; expression is induced by providing a source of T7 RNA polymerase in the host cell. T7 RNA polymerase is so selective and active that almost all of the cell's resources are converted to target gene expression; the desired product can comprise more than 50% of the total cell protein a few hours after induction. Another important benefit of this system is its ability to maintain target genes transcriptionally silent in the uninduced state. Target genes are initially cloned using hosts that do not contain the T7 RNA polymerase gene, thus eliminating plasmid

instability due to the production of proteins potentially toxic to the host cell....Two types of T7 promoter and several hosts that differ in their stringency of suppressing basal expression levels are available, providing great flexibility and the ability to optimize the expression of a wide variety of target genes" (page 3). The manual further teaches that the pET-28a(+) vector contains an N-terminal histidine tag for simple affinity purification of the recombinant protein (see Table on page 6).

Regarding preparation of a pET expression construct the manual presents the following procedure (see page 15 for a flowchart and specific recommendations on pages 18-26):

(a) prepare the pET vector by restriction enzyme digestion and dephosphorylation (step 1 on page 15; see also pages 18-19, where more details of the restriction enzyme digestion are presented and calf intestinal alkaline phosphatase is used to dephosphorylate the vector)

(b) prepare the insert DNA by restriction digestion (step 2 on page 15; see also page 19 for specific details of the digestion and isolation of the digested fragment)

(c) ligation of the insert and vector followed by transformation into the appropriate host (step 3 on page 15; see also pages 20-22 for specific details of the ligation and transformation)

(d) identify positive clones by colony PCR, miniprep, sequencing or in vitro transcription/translation (step 4 on page 15; see also pages 22-26 for specific details)

The manual teaches that suitable hosts for cloning include the following recA and endA deficient strains: NovaBlue, JM109, and DH5a (page 8), but does not teach the INVαF' strain. Also the pET system manual does not teach digestion using the BamHI and XhoI restriction enzymes.

Novagen catalog No. TB074 teaches the vector map of the pET-28a(+) vector. This vector contains BamHI and XhoI sites in the cloning region (see page 1).

The 1997 Invitrogen catalog (page 85) teaches INVαF' competent E. coli cells. This strain is recA and endonuclease A deficient. Invitrogen teaches that these cells "are ideal for propagating plasmids and cDNA library construction. They allow stable replication of high-copy plasmids" (page 85). The genotype offers the following features: (a) blue-white screening, (b) reduction of homologous recombination of transformed plasmids (recA), and (c) increased quality of plasmid DNA preparations (endA1) (see page 85).

It would have been prima facie obvious for one of ordinary skill in the art at the time of invention to construct expression plasmids of the human SMN gene in the pET-28a(+) expression system. Melki taught expression constructs of the human SMN gene using a variety of different vectors, including bacterial plasmid vectors (column 13, lines 39-42, cited above), but did not specify a preferred bacterial expression vector system. As discussed above, the pET system offered rapid, stable, high-yield expression of recombinant proteins modified with a histidine tag for simple affinity purification (see above citation). The ordinary practitioner would have been motivated to generate SMN expression constructs as suggested by Melki using the pET-28a(+) vector taught by Novagen in order to rapidly produce a large amount of recombinant protein, suitable for simple, often one-step affinity-based purification. Since the Novagen product information sheets provided specific guidance as to the method of producing the expression construct (see above), and the vector map taught that the BamHI and XhoI sites were

available in the cloning site, the ordinary practitioner would have expected a reasonable level of success in producing the claimed expression constructs. Regarding the choice of cloning host cells, the Novagen pET system manual expressly taught that the major requirement for the host cell is a strain deficient in recA and endA activity, and further taught that in addition to the exemplary E. coli strains mentioned on page 8, other equivalent strains could be used. Since the Invitrogen catalog taught that INVαF' cells were recA and endA deficient and useful for routine cloning applications (see above), the ordinary practitioner would have been motivated to substitute any suitable recA endA deficient cell line in the method. In short, the claimed method is directed to obtaining an expression construct of a known gene (SMN) in a known expression system (pET) using well-known, standard methods, and therefore is prima facie obvious in light of the prior art teachings cited above.

Conclusion

No claims are currently allowable.

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